are so selected as to immobilize moieties **46** without appreciably affecting the reactivity (e.g., the affinity and avidity) of the moiety for the complementary portion of the complex. In a preferred embodiment, fiber **12** is of glass or quartz, coupling sites **44** are the reactive groups of a silyl compound such as 3-aminopropyltrimethoxysilane, and moieties **46** are an antibody such as immunoglobulin G (IgG). This particular combination of solid phase, coupling site **44** and moiety **46** may be bound through the antibody's carboxyl terminations, thereby leaving the antibody's antigen reactive amino terminations free.

The methods of preparing the glass surface of fiber **12**, of attaching the silyl compound thereto, and of covalently bonding an antibody to the glass through the silyl coupling, are described by Weetall (U.S. Pat. No. 3,652,761), where may also be found a description of other silyl compounds and the methods by which carboxyl, amino, and other reactive groups of antibody or antigen (or their fragments) may be covalently bound to various inorganic materials. It should be noted that an extensive art for immobilizing antigens or antibodies to polymers also exists, and those skilled in the art will understand that coupling sites **44** for antigen or antibody might be provided on polymeric fibers also. Thus, for instance, if fiber **12** is of nylon (polyamide), the coupling may be in the form of the substitution of an appropriate radical for the hydrogen bound to the polymer's amino groups. It should be noted that coupling sites **44** may also incorporate spacer groups, as are well known in the art, to insure sufficient separation between fiber **12** and moieties **46** as to minimize steric hindrance of the antibody-antigen binding process. For example, coupling sites **44** might include a polyethylene chain, as for example in the case of 1,6 diaminohexane or 6 aminohexanoic acid bound to fiber **12** through a peptide bond and respectively providing a free primary amino and a free carboxyl group for covalent binding to the carboxyl or amino termination of a protein moiety **46**. Either of these coupling materials provide a 6-carbon chain between terminations, thereby spacing moiety **46** from fiber **12** by the corresponding distance. Similar appropriate coupling and spacer materials are well known in the arts of both immunoassay and affinity chromotography.

In a preferred embodiment, fiber **12** is provided with moiety **46** having occupied binding sites, as indicated at index numerals **46C**, the moieties being in part provided with attached tagged complement **50** for competition immunoassays. Thus, in one embodiment moiety **46** is an antibody, and a preloading of tagged antigen or hapten is incorporated into the coating of fiber **12**. Each of the tagged components **50** is provided with a predetermined quantity of fluorophore **52**, thereby providing a tag. The particular fluorescing compound of interest for tagging include fluorescein, tetramethylrhodamine, rare earth chelates, and the like. Methods for linking fluorescent tags to proteins are well known in the art, and many of the commercially available fluorescing compounds have groups for linking to proteins. Preferably, for competition assay, a fixed portion of coupling sites **44** are provided with a immunologically inert protein **56**, such as albumin. The coating can be made to have a fixed surface composition by using absorption phenomena, as follows. For a coating solution prepared with appropriate concentration of the reagents, mere immersion of a fiber activated with the proper surface binding groups **44** will produce a surface monolayer of chemically bound protein. The proportion of, immunoglobulin to inert protein in this layer will be given by (but not identical to) their proportion in the solution. Any partial filling of the immunoglobulin active sites with tagged antigen will, of course, be maintained at the level in the solution. After dipping, the fiber is removed from the coating solution. To prevent the adhering liquid layer from entraining additional reagent, the fiber is then quickly washed before further evaporation can occur. The protein layer, being covalently bound, will not be dislodged by this process. In order to prevent binding of more than one layer of protein, the bifunctional reagent must not alter the net charge of the protein (this can be controlled by adjusting the pH of the coating solution) and not have too long a spacer arm.

Kit **6** is intended for use with fluorimeter **7** (FIG. **4**) which is chosen to excite and accept fluorescence from the tagged component of the reagent. A suitable fluorimeter is described in the aforementioned U.S. Pat. No. 4,447,546, wherein may also be found a description of its operation. Importantly, as described in the latter patent, the fluorimeter preferably reads the fluorescent emission propagated back toward that end of the fiber at which the excitation radiation was introduced.

Kit **6** of the present invention is used much as in either of the other patent applications hereinbefore referred to. However, and importantly, in the present invention, the volume of sample used in a determination is not necessarily limited by the interspatial volume between fiber **12** and tube **14**.

In use, the preferred embodiment of the apparatus of the present invention requires first that a sample be drawn into syringe **8** which is then connected, as by a Luer lock to kit **10** as in FIG. **1**, i.e, syringe **8** is placed in syringe pump **9** and kit **6** is connected to fluorometer **7**. Syringe pump **9** is now activated, forcing the sample in syringe **8** through kit **6** at a flow rate as will be described. Pump **9** thus constitutes means for controlling the flow rate so that the dwell time of the sample **43** opposite activated region **36** is similar to the time required to thoroughly scavenge the volume between fiber **12** and tube **14**. Faster flow rates will increase the sensitivity of the system but will tend to decrease the efficiency of use of the sample because of incomplete scavenging. The sample flow through the interspace between fiber **12** and tube **14** should completely fill that interspace and preferably keep it filled during the entire assay. As taught in U.S. Pat. No. 4,447,546, fluorimeter **7** is configured so as to illuminate end face **24** of fiber **12** within the cone angle defined by the numerical aperture of the fiber. This radiation is consequently propagated within fiber **12** at or above the critical angle (as indicated by ray **54** in FIG. **3**) and multiply totally internally reflected along the length of the fiber. As a result, an evanescent wave is produced in fluid **43** adjacent the fiber during the flow of the sample part activated region **36**.

Competitive binding of tagged components **50** and untagged components **54** to moieties **46** attached to the fiber results in fluorescently tagged complexes **46C** in proportion to the relative concentration of tagged to untagged components. Excited by the evanescent wave, the tagged complexes **46C** fluoresce. A portion of the fluorescent emission tunnels into the fiber, propagating within the fiber along paths exceeding the critical angle, as indicated, for instance, by ray **56** in FIG. **3**. One-half or more of this totally reflected fluorescence emission